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Mechanisms of biotin-regulated gene expression in microbes

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ABSTRACT

Biotin is an essential micronutrient that acts as a co-factor for biotin-dependent metabolic enzymes. In bacteria, the supply of biotin can be achieved by *de novo* synthesis or import from exogenous sources. Certain bacteria are able to obtain biotin through both mechanisms while others can only fulfill their biotin requirement through *de novo* synthesis. Inability to fulfill their cellular demand for biotin can have detrimental consequences on cell viability and virulence. Therefore understanding the transcriptional mechanisms that regulate biotin biosynthesis and transport will extend our knowledge about bacterial survival and metabolic adaptation during pathogenesis when the supply of biotin is limited. The most extensively characterized protein that regulates biotin synthesis and uptake is BirA. In certain bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, BirA is a bi-functional protein that serves as a transcriptional repressor to regulate biotin biosynthesis genes, as well as acting as a ligase to catalyze the biotinylation of biotin-dependent enzymes. Recent studies have identified two other proteins that also regulate biotin synthesis and transport, namely BioQ and BioR. This review summarizes the different transcriptional repressors and their mechanism of action. Moreover, the ability to regulate the expression of target genes through the activity of a vitamin, such as biotin, may have biotechnological applications in synthetic biology.

1. Introduction

Biotin (vitamin H or B7) is an important micronutrient that functions as a cofactor for biotin-dependent enzymes.[1] These include the biotin-dependent carboxylases, decarboxylases and transcarboxylases, all of which are found in the microbial world. In the prototypical bacteria *Escherichia coli*, there is a single biotin-dependent enzyme, namely acetyl CoA carboxylase, that catalyzes the first committed step in the fatty acid biosynthesis pathway.[2,3] Other examples of biotin-dependent enzymes commonly found in prokaryotes include pyruvate carboxylase responsible for replenishing the TCA cycle with oxaloacetate,[4] and propionyl CoA carboxylase required for the metabolism of certain amino acids and fatty acids.[5] Micro-organisms, plants and some fungi are able to synthesize biotin *de novo* as well as importing it from their environment through the action of a biotin transport system. In contrast, humans and other mammals are biotin auxotrophs and rely solely on uptake from external sources, such as intestinal microflora or the diet.[6] This genetic difference in biotin metabolism between humans and microbes provides potential drug targets for new antibiotic discovery (reviewed[7]). The biotin synthesis pathway is well characterized in *E. coli* and *Bacillus subtilis* and has recently been reviewed.[8] In many bacteria the genes that encode the biotin biosynthetic enzymes are often clustered into an operon known as the *bio* operon.[9] Briefly, the synthetic pathway commences with L-alanine and S-adenosyl-L- methionine being introduced into pimeloyl-ACP by the activities of 7-keto-8-aminopelargonic acid synthase (encoded by *bioF*) and 7,8-diaminopelargonic acid synthase (encoded by *bioA*), respectively, to generate 7,8-diaminopelargonic acid. Dethiobiotin synthetase (encoded by *bioD*) and biotin synthase (encoded by *bioB*) then catalyze the closure of the ureido and thiophane heterocycles, respectively, liberating biotin.

The *de novo* synthesis of biotin is metabolically costly, requiring 20 equivalents of ATP for each molecule of biotin and the activities of at least 4 metabolic enzymes.[10] Therefore, transcriptional regulation of the biotin biosynthetic enzymes needs to be

Abbreviations: BCCP, biotin carboxyl carrier protein; BirA, biotin retention protein A; BPL, biotin protein ligase; *Ec*BirA, *Escherichia coli* BirA; *Sa*BirA, *Staphylococcus aureus* BirA.

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tightly controlled. In the model bacteria *E. coli*, the balance of biotin demand versus supply is maintained through the action of the biotin retention protein A (BirA); a bi-functional protein that is not only a transcriptional repressor but also serves as the biotin ligase that catalyzes the attachment of biotin onto the biotin-dependent carboxylases. In other microorganisms, such as *Corynebacterium glutamicum* and *Agrobacterium tumefaciens*, there is no BirA homolog to regulate biotin synthesis and transport. Instead, alternative DNA-binding proteins perform this function, namely BioQ and BioR respectively. The mechanisms by which BirA, BioQ and BioR regulate biotin biosynthesis and transport will be discussed in this review.

2. BirA is a bi-functional protein

BirA serves as both a transcriptional repressor and the enzyme responsible for protein biotinylation (outlined in Fig. 1). As both biotin ligase and transcriptional repressor activities are intimately linked, we provide an overview of both functions as background for the reader to understand the sophistication of this elegant system. Protein biotinylation is achieved through a conserved, two-step reaction mechanism that is catalyzed by biotin protein ligase (BPL) in all organisms. In the first partial reaction biotin and ATP are required to form biotinyl-5′-AMP that serves as both the reaction intermediate for protein biotinylation and corepressor for transcriptional regulation. The BirA: biotinyl-5′-AMP (holo) enzyme can then adopt one of two different fates. When the cellular demand for biotin is low holo BirA can dimerize and bind DNA where it functions as the transcriptional repressor of the biotin biosynthesis operon, thereby inhibiting the synthesis of more biotin. In contrast, in the presence of substrate requiring biotinylation the holo BirA functions as a biotin ligase. Here BPL recognizes and binds to a biotin carboxyl carrier protein (BCCP) present in the receiving enzyme that contains the lysine residue targeted for biotinylation.[11] Protein biotinylation is an example of a post-translational modification that is performed with exquisite specificity. For example, the *E. coli* biotin ligase (BirA) modifies just one of the >4000 different proteins in the bacterial cell.[12] Moreover, the biotin cofactor is covalently attached onto the side chain of one single, specific target lysine residue present in the active site of biotin-dependent enzymes. BPLs from a wide variety of species are able to modify BCCP from unrelated organisms,[13–15] highlighting how highly conserved both the catalytic mechanism and the protein:protein interactions between enzyme and substrate have remained throughout evolution. The possible mechanisms through which BirA can switch between its two functions are described later in this review.

All BPLs contain a conserved 2-domain catalytic core responsible for biotinyl-5′-AMP synthesis and protein biotinylation.[16] The greatest divergence between the BPLs is in their N-terminal regions (see Fig. 2A). Class I BPLs are composed only of the conserved catalytic module that is required for protein biotinylation. Hence, these are mono functional enzymes. X-ray crystal structures of Class I BPLs have been reported for *Mycobacterium tuberculosis*[19] and *Pyrococcus horikoshii*.[21] In contrast, the Class II BPLs are truly bi-functional having both biotin ligase and transcriptional repressor activities due to an N-terminal DNA binding domain. BirA from *E. coli* is the most extensively studied representative of a Class II BPL, having been the subject of structural, genetic and biophysical studies (reviewed[22,23]).

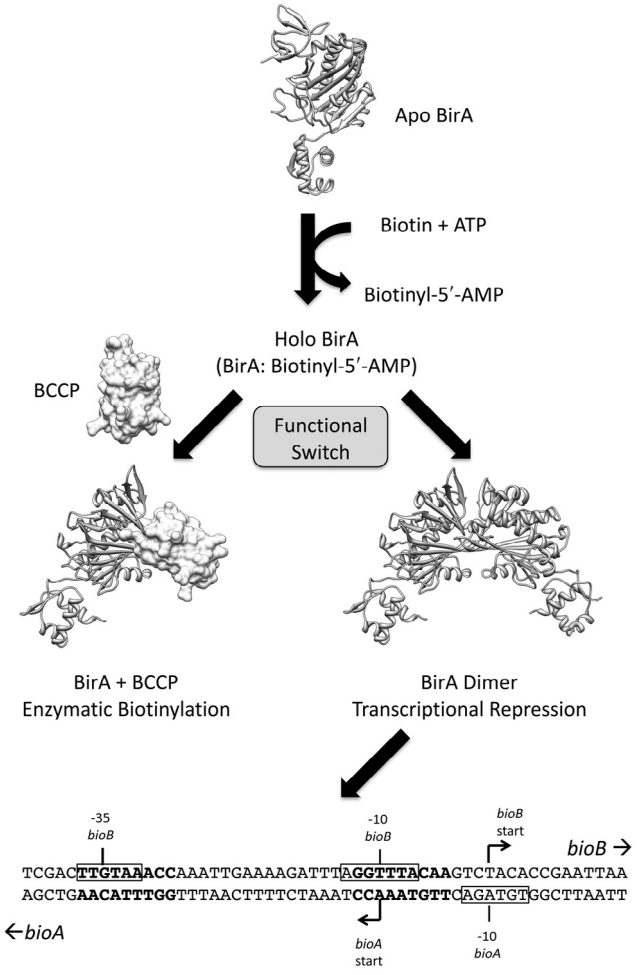

Fig. 1. Bifunctional BirA from *Escherichia coli*. The schematic shows the two alternative functions for the protein. The *bioO* sequence from the biotin biosynthetic operon is shown below, with the BirA binding sites in bold text and the –10 and –35 sequences boxed.

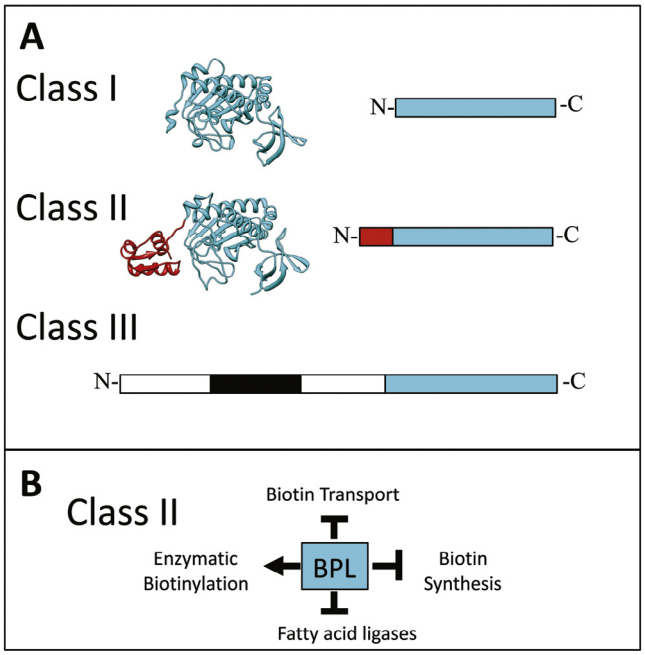

Fig. 2. Biotin Protein Ligase. (A) The relative sizes of the three structural classes of BPLs are shown. The conserved catalytic region is depicted in blue, the DNA binding domain of Class II enzymes in red and the proof reading domain in human BPL is boxed black.[17,18] The structures of BPLs from *M. tuberculosis* [PDB 3RUX[19]] and *E. coli* [PDB 2EWN[20]] are highlighted. (B) Schematic overview showing the single protein model of protein biotinylation and transcriptional regulation in Class II BPLs.